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STUDIES OF FLUOROMETRIC ASSAY PROCEDURES
FOR LYSERGIC ACID DIETHYLAMIDE

BY

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Studies of the available fluorometric assay procedures for LSD-25 are described for possible clinical application. Variability of plasma 'blank' background fluorescence values were found to prohibit the use of standard fluorometric procedures without modification. A little known fluorometric procedure is described, which minimizes this problem and maintains the sensitivity of the assay at the nanogram level. Modifications of this method are suggested which could increase the sensitivity of this method to the subnanogram level.

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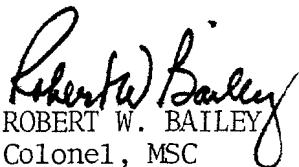


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STUDIES OF FLUOROMETRIC ASSAY PROCEDURES FOR LYSERGIC ACID DIETHYLAMIDE

INTRODUCTION

The abuse of the hallucinogenic agent lysergic acid diethylamide (LSD-25) in man is well known. The decrement in performance levels, perceptual changes, mood changes and hallucinations which occur in individuals under the influence of LSD-25¹⁻³ constitute a great hazard in aviation safety. This hazard is compounded by the existence of delayed reactions (flashbacks) following the use of the drug.⁴

A biochemical method to identify LSD-25 in biological materials would be of great value in confirmation of diagnosis and post-accident investigation. Furthermore, if a reliable and sensitive clinical procedure could be developed it would be of great value in drug abuse screening programs.

This report deals with preliminary, in vitro studies of the available fluorometric assay procedures for LSD-25. A little known fluorometric assay is described which minimizes problems of variability in plasma 'blanks' and maintains the sensitivity of the assay at the nanogram level.

EXPERIMENTAL SECTION

It is well known that LSD-25 doses as low as 1 μ g per kg are pharmacologically effective in man. Furthermore, it has been shown that the level of unchanged LSD-25 in blood plasma drops to 1-2 ng/ml, between 6 to 8 hours after the administration of a 1-2 μ g per kg dosage, either intravenously⁵ or orally,⁶ to human subjects. The low dosage and rapid transformation of LSD by the body has required the development of specific and sensitive methods for the detection of the drug in body tissues. The most widely known techniques for the determination of LSD-25 at the nanogram level are the procedures of Axelrod, *et. al.*⁶ and Aghajanian and Bing.⁵ Both procedures are based on the fluorescence characteristics of the drug and are very similar in methodology.

In the present in vitro study, the procedures utilized for the extraction of LSD-25 from human plasma specimens are based on both of the above methods. LSD tartrate, Sandoz, Batch #69003 was obtained from the FDA-NIMH Psychotomimetic Agents Advisory Committee, Center for Studies of Narcotic and Drug Abuse. The drug was added to blood plasma specimens prior to initiation of the extraction procedure. Plasma specimens were obtained freshly

or from expired ACD Whole Blood. All glassware concerned with the fluorescence assay were cleaned with nonfluorescent soap (Drene), rinsed and washed well with distilled water. All organic solvents were spectral grade reagents. All procedures involving LSD-25 were carried out in subdued light. Five milliliters of plasma were placed in a 50 ml glass stoppered centrifuge tube and saturated with NaCl (\sim 2g). One-half milliliter of 1N NaOH was added to the salt saturated plasma and the resulting mixture was extracted with 20 ml of n-heptane containing 2% isoamyl alcohol for 30 minutes on an Extractomatic shaker (Virtis). After extraction and centrifugation, 18 ml of the heptane phase was transferred to a glass centrifuge tube containing 1.5 ml of 0.004 N HCl. The resulting mixture was shaken for 15 minutes (Extractomatic) and centrifuged. The heptane phase was removed by suction and discarded. One ml of the acid phase was placed in a quartz cuvette and the fluorescence of the solution was measured with an Aminco-Bowman spectrophotofluorometer. Excitation and fluorescence wavelengths were 325 and 432 μm respectively.

When it was desired to utilize UV irradiation as described by Upshall and Wailling,⁷ a Stroblite 100 W UV lamp was used. The extracted LSD samples, in 0.004 N HCl, were irradiated in their quartz cuvettes for a period of three hours at which time the fluorescence was determined a second time. The fluorescence due to LSD by this method is the difference in fluorometer readings before and after UV irradiation.

RESULTS AND DISCUSSION

Sensitivity of LSD-25 to Light:

Initial studies of the fluorescence of LSD-25 indicated that the emission decreased with exposure to activating light. Axelrod, et. al.,⁶ found similar results in his studies. Figure 1 describes the effect of excitation light on the fluorescence of LSD-25 in 0.004 N HCl. The emission decreased linearly for the first five minutes and nonlinearly for the remaining 25 minutes. After 30 minutes of exposure to excitation light there was little change in fluorescence. Since excitation light was shown to adversely affect the fluorescence of LSD-25, all studies were carried out in subdued light and Slit A of the spectrophotofluorometer was used as a shutter to initiate exposure to excitation light. Fluorescence readings were recorded immediately after opening the shutter.

Linearity of the Basic Fluorometric Method:

To five samples of plasma, LSD-25 was added over the concentration range 0 to 10 ng/ml. The samples were extracted as described in the experiment without UV irradiation. This extraction procedure, as previously stated,

**THE EFFECT OF EXCITATION LIGHT ON THE FLUORESCENCE
OF LSD - 25**

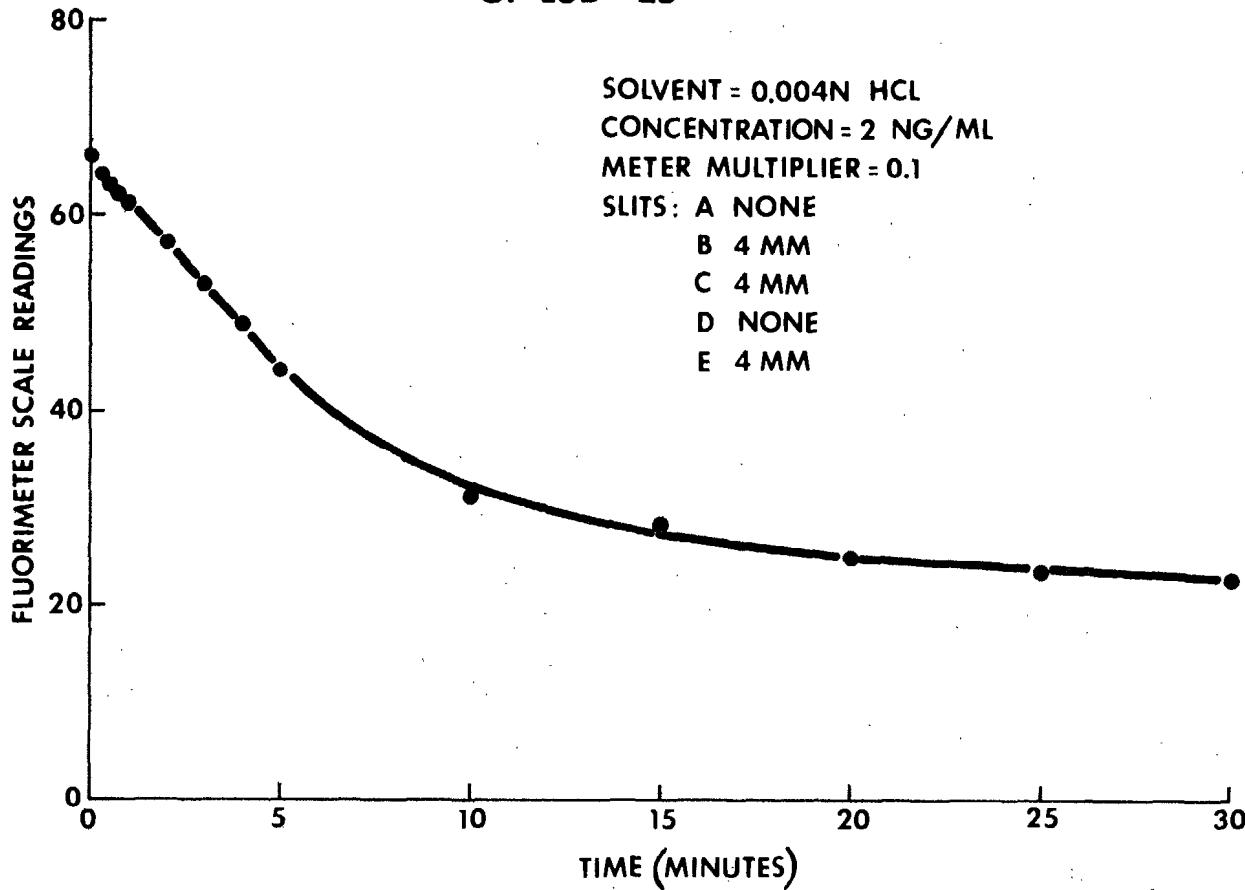


Figure 1

was basically that of Axelrod, et. al.,⁶ and Aghajanian and Bing.⁵ Fluorometer scale readings were plotted as a function of the concentration of LSD-25 in the starting material (See Figure 2). The results of this study indicated that the linearity of the assay was good. With careful technique as little as 1 ng LSD-25/ml could be determined.

Background Fluorescence:

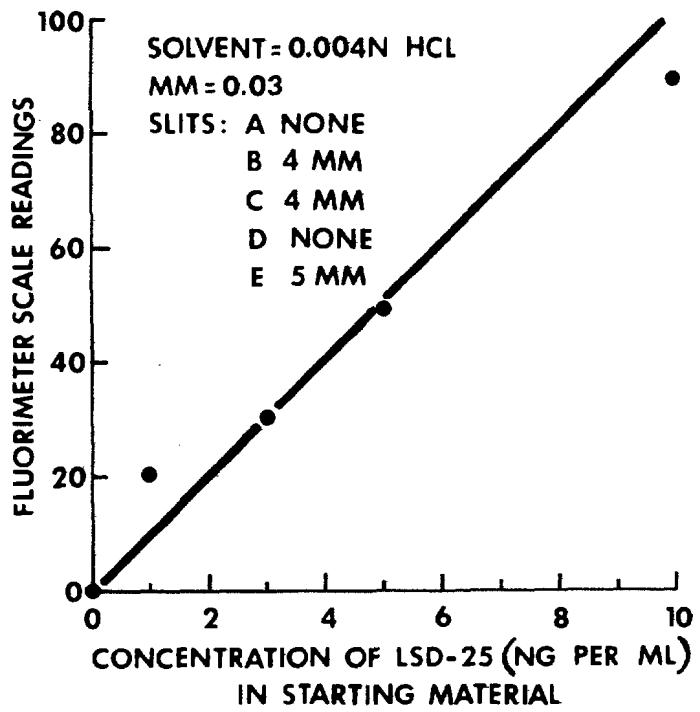
One of the problems which can occur in the adaption of a highly sensitive fluorometric assay procedure to use as a clinical method is a high or variable background fluorescence. When the fluorescence due to LSD is small relative to the background it becomes more difficult to assay low concentrations of the drug and the sensitivity of which the assay suffers. If the background fluorescence is variable the sensitivity also suffers in that low concentrations may fall within the normal variation for background fluorescence. In an attempt to assess the magnitude of this problem, the background fluorescence (in the absence of added LSD-25) of 18 samples of plasma was determined by the methods of Axelrod, et. al.,⁶ and Aghajanian and Bing⁵ as previously described. Fluorescence readings were obtained over the range 0.59 to 3.20% Transmittance, with an average at 1.85% T. Studies of plasma to which LSD had been added gave readings varying from 1.62 to 2.43% T for the range of concentrations, 1 to 5 ng LSD-25/ml. It is, therefore, impossible to determine low concentrations of LSD-25 (under about 7 ng/ml) by the above described method, since the low fluorescence readings due to LSD fall within the range of normal plasma background fluorescence variation.

Studies of the Upshall and Wailling Method for the Determination of LSD-25:

In an attempt to circumvent the problems caused by background fluorescence variation, the assay of LSD-25 was undertaken by the method of Upshall and Wailling.⁷ The procedure, as described in the Experimental Section above, is based on the fact that UV irradiation catalyzes the hydration of LSD to the nonfluorescent lumiderivative, in which a molecule of water is added across the C₉ - C₁₀ double bond. Thus, the difference in fluorescence of plasma extracts before and after intense UV irradiation is a measure of LSD concentration.

Figures 3A and B describe the differences in fluorometer scale readings before and after UV irradiation as a function of the concentration of LSD-25 in the plasma samples. The experiment described by Figure 3A utilized "aged" LSD which had been prepared in 0.004N HCl three weeks prior to the experiment. The experiment described by Figure 3B utilized freshly prepared solutions of the drug. The results of these studies

LINEARITY OF THE ASSAY FOR LSD-25*



* BY THE METHOD OF AXELROD ET AL(6) AND AGHAJANIAN AND BING(5).

Figure 2

LINEARITY OF THE ASSAY FOR LSD-25*

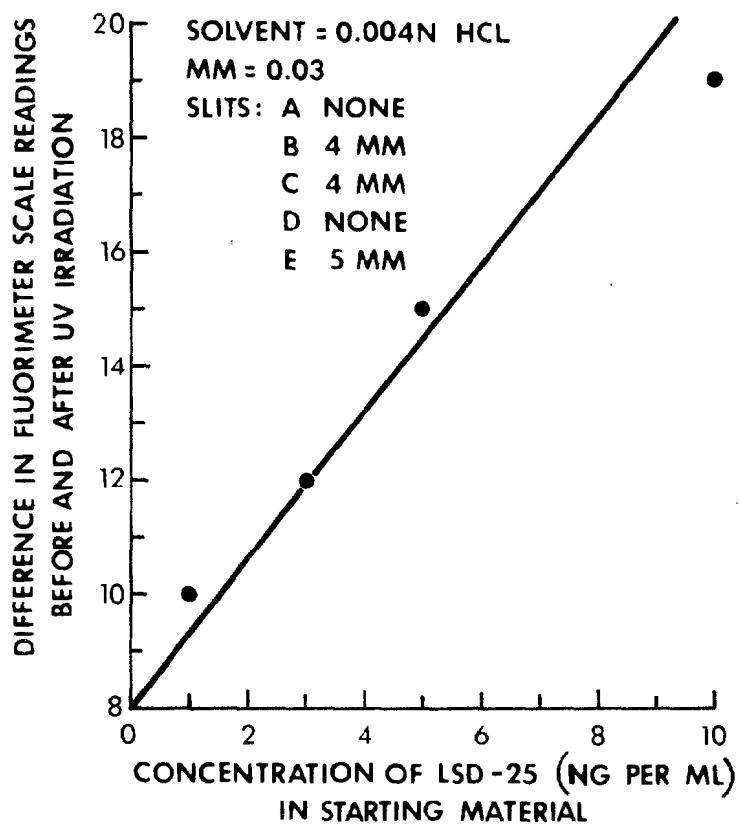


Figure 3

A

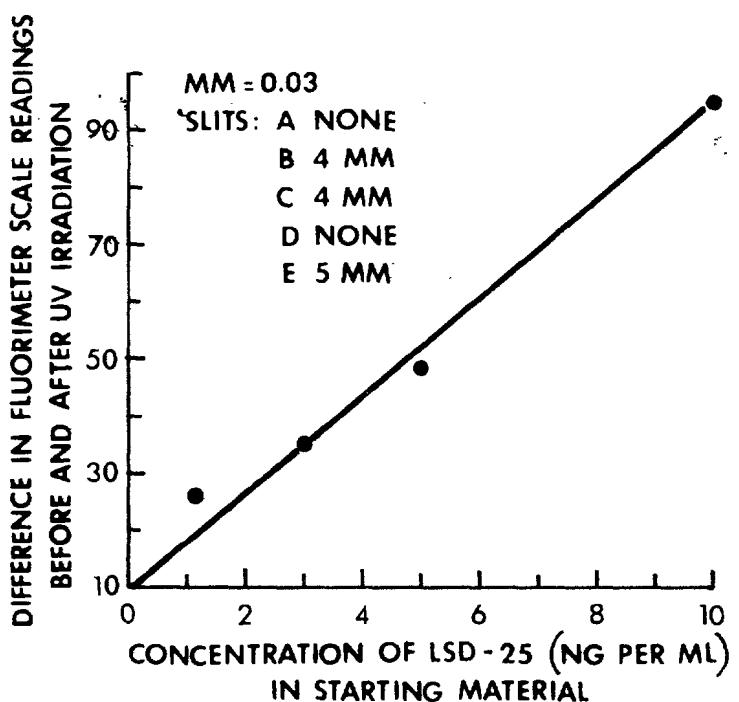


Figure 3

B

* BY THE METHOD OF UPSHALL AND WAILLING (7).

indicated that the assay was linear over the concentration range 0.0 to 10.0 ng/ml. As indicated in Figures 3A and B the line describing the differences in fluorometer readings vs. concentration did not pass through the origin. This can be explained by the fact that plasma 'blanks' (without added LSD) showed a decrease in fluorescence upon exposure to UV irradiation. The difference in readings for the blank was fairly constant (8-10 T) for the two experiments described. It is possible that this effect may be the result of excessive UV irradiation or heating, or may be within experimental error for the method. Upshall and Wailling⁷ stated that plasma was resistant to the UV treatment and their data did not indicate any effect. Further study could resolve this problem. With freshly prepared LSD (Figure 3B) less than 2 ng/ml of the drug could be detected by the above method without the use of a plasma 'blank', assuming that the blank would have given a relatively constant decrease in fluorescence due to UV irradiation.

It is possible that the sensitivity of this method could be increased further by: (1) increasing the volume of plasma extracted; (2) increasing the number of extractions with n-heptane/isoamyl alcohol, while keeping the total volume used the same; and/or (3) decreasing the volume of the 0.004N HCl utilized in the final extraction step. However, the method as presently described could have some value in toxicological examinations where the plasma concentration of LSD-25 is suspected to be approximately 1.5 ng/ml or more. If a relatively large sample of plasma (approximately 20 ml) was available, known concentrations of LSD-25 could be added to aliquots of the plasma and by the use of the Upshall and Wailling method⁷ the LSD added could be assayed. By plotting the difference in fluorescence of the extracted LSD, before and after UV irradiation, as a function of added LSD, the concentration of endogenous LSD in the plasma could be extrapolated.

CONCLUSIONS

Studies of the known fluorometric assay procedures for LSD-25 have been described for possible clinical/toxicological application. This preliminary investigation has indicated that the Upshall and Wailling⁷ method for the fluorometric assay of LSD-25 is an acceptable method for the determination of nanogram levels of the drug. It is likely that the sensitivity of this method could be increased to subnanogram level.

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